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Aerosolization of fungi, (1→3)-β-D glucan, and endotoxin from flood-affected materials collected in New Orleans homes ☆

Atin Adhikari [a], Jaehee Jung [a,b], Tiina Reponen [a], Jocelyn Suzanne Lewis [c], Enjoli C. DeGrasse [c], L. Faye Grimsley [c], Ginger L. Chew [d], Sergey A. Grinshpun [a,*]

[a] Center for Health-Related Aerosol Studies, Department of Environmental Health, University of Cincinnati, P.O. Box 670056, 3223 Eden Avenue, Cincinnati, OH 45267-0056, USA
[b] Department of Mechanical Engineering, Korea Advanced Institute of Science and Technology, Daejeon 305-701, Republic of Korea
[c] Department of Environmental Health Sciences, Tulane University, New Orleans, LA 70112, USA
[d] Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, New York, NY 10032, USA

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ABSTRACT

Standing water and sediments remaining on flood-affected materials were the breeding ground for many microorganisms in flooded homes following Hurricane Katrina. The purpose of this laboratory study was to examine the aerosolization of culturable and total fungi, (1→3)-β-D glucan, and endotoxin from eight flood-affected floor and bedding materials collected in New Orleans homes, following Hurricane Katrina. Aerosolization was examined using the Fungal Spore Source Strength Tester (FSSST) connected to a BioSampler. Dust samples were collected by vacuuming. A two-stage cyclone sampler was used for size-selective analysis of aerosolized glucan and endotoxin. On average, levels of culturable fungi ranged from undetectable (lower limit = 8.3×10^4) to 2.6×10^5 CFU/m^2; total fungi ranged from 2.07×10^5 to 1.6×10^6 spores/m^2; (1→3)-β-D glucan and endotoxin were 2.0×10^3–2.9×10^4 ng/m^2 and 7.0×10^2–9.3×10^4 EU/m^2, respectively. The results showed that 5–15 min sampling is sufficient for detecting aerosolizable biocontaminants with the FSSST. Smaller particle size fractions (<1.0 and <1.8 μm) have levels of glucan and endotoxin comparable to larger (>1.8 μm) fractions, which raises additional exposure concerns. Vacuuming was found to overestimate inhalation exposure risks by a factor of approximately 10^2 for (1→3)-β-D glucan and by 10^3–10^4 for endotoxin as detected by the FSSST. The information generated from this study is important with respect to restoration and rejuvenation of the flood-affected areas in New Orleans. We believe the findings will be significant during similar disasters in other regions of the world including major coastal floods from tsunamis.

1. Introduction

During last decade many countries of the world have seen natural disasters from floods. The unprecedented disasters caused by Hurricanes Katrina and Rita in 2005 disrupted the public health and medical infrastructures in New Orleans and created many difficult environmental health challenges. Standing water and sediments remaining in flooded areas were breeding grounds for various microorganisms, including fungi and bacteria that could become airborne and be inhaled; in turn, these exposures may increase the incidence of lung disease (American Lung Association, 2008), among other health effects. Fungi have been associated with allergic respiratory disease, especially asthma (Douwes et al., 2003). Total fungi, including spores from both culturable and non-culturable species, may be as allergenic or toxigenic as the culturable fungi (Flannigan, 1997). Viable fungi can release allergens during germination (Green et al., 2003) and may cause mycotic infections in immunocompromised subjects (Burge, 2001; Eduard, 2003). The association of (1→3)-β-D glucan (a polyglucose molecule comprising up to 60% of the cell wall of most fungal taxa) with dry cough, phlegmy cough, hoarseness, and atopy has been reported in indoor environments (Rylander et al., 1998; Rylander, 1999). Bacterial growth in flooded homes can be a significant source of endotoxin, which is a lipopolysaccharide component of the outer membrane of Gram-negative bacteria. Inhaled endotoxin can contribute significantly to the induction of airway inflammation and dysfunction (Pirie et al., 2003).

There are significant public health concerns about exposure to airborne microorganisms and the associated respiratory health effects; however, the scientific knowledge regarding the aerosolization of microorganisms in field conditions, including the

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* Corresponding author. Fax: +1 513 558 2263.
E-mail address: sergey.grinshpun@uc.edu (S.A. Grinshpun).

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flood-affected buildings, is still insufficient. While air sampling is currently deemed to be the most representative method of determining respiratory exposure, there are several problems with traditional air sampling methods. Most commercially-available bioaerosol sampling instruments (e.g., Burkard, Andersen, Air-O-Cell) cannot be used for assessing long-term exposure to airborne microorganisms, as their sampling time is limited to 3–20 min. At the same time, the spore concentrations have wide temporal variations, particularly in mold-contaminated homes (Hyvärinen et al., 2001). In addition, each sampling method has limitations, and a standard unanimously-accepted bioaerosol sampling method is not yet available.

Information about airborne microorganisms that is assessed by conventional air sampling during specific time intervals may not be representative of potential exposure levels; it may not be able to reliably detect microbial colonization and their successive aerosolization from contaminated surfaces (Horner, 2003). Moreover, the release of microorganisms from surfaces does not necessarily occur during air sampling. Release of certain microorganisms may be sporadic, even with no disturbance of the surfaces and especially when it is caused by movements of occupants or workers engaged in clean-up activities. It has been widely recognized that a reliable technique is required to directly assess the aerosolization potential of microorganisms growing on contaminated surfaces in indoor environments. Direct source evaluation techniques, such as bulk sampling, surface sampling (e.g., swab and tape sampling) and dust sampling by vacuuming, allow the investigators to collect extensive information about the source. However, the aerosolization potential of contaminants from their sources cannot be measured with these methods, as they collect both aerosolizable contaminants as well as those that adhere to the deeper surface of substrates; thus, they are unlikely to become aerosolized under normal circumstances. Therefore, the results may not adequately represent or predict the exposure risks to aerosolized microorganisms from a contaminated material. The problem has been addressed in this study by utilizing a Fungal Spore Source Strength Tester (FSSST), an inexpensive and portable device previously developed and evaluated by our group. The device was found suitable for aggressive sampling of releasing potentially aerosolizable fungal spores from mold-contaminated sources (Grinshpun et al., 2002; Sivasubramani et al., 2004a, b; Niemeier et al., 2006; Seo et al., 2008). This study utilized the FSSST to gain a fundamental understanding of the aerosolization of the potential moisture-related microbiological hazardous agents (culturable and total fungi, (1→3)-β-D glucan, and endotoxin).

In addition to these three biocontaminants, dust mite allergens were also analyzed in selected samples. There were two reasons behind these tests. First, we anticipated that lower levels of aerosolized fungi, observed for several materials, can be influenced by the presence of dust mites, which affect the fungal growth surfaces. Second, dust mites can use fungal mycelium as food, thus facilitating fragmentation and subsequent aerosolization of small particles containing relatively higher amount of (1→3)-β-D glucan. There are reports of interactions between domestic dust mites and fungi in indoor environments (Asselt, 1999) and antagonistic and mutual ecological relationships between xerophilic fungi (found prevalent in this study) and dust mites at higher relative humidity (Lustgraaf, 1978).

Another important focus in this study was to determine the concentrations of aerosolized biocontaminants size-selectively. Bioaerosol particles vary in size depending upon the microorganism, the aerosolization mechanism, and environmental parameters (Reponen et al., 1994, 1996; Meklin et al., 2002; Górny et al., 2002; Lindsley et al., 2006; Wang et al., 2007). Furthermore, fine particles may be more damaging to human respiratory health than larger ones, regardless of their low mass, as they penetrate deeper into airways (Ferin et al., 1990; Oberdorster, 1996). The National Institute for Occupational Safety and Health (NIOSH) has recently developed a two-stage cyclone sampler for size-selective personal sampling of bioaerosols (Lindsley et al., 2006). In this study, this sampler was used to investigate aerosolized (1→3)-β-D glucan and endotoxin in three size fractions.

To summarize, the purposes of this laboratory study were: (i) to determine aerosolization of culturable and total fungi, (1→3)-β-D glucan, and endotoxin from eight different flood-affected materials; (ii) to investigate aerosolization of (1→3)-β-D glucan and endotoxin with size-selective sampling; and (iii) to compare the levels of these biocontaminants measured by FSSST and a vacuum cleaner.

2. Materials and methods

2.1. Collection of flood-affected materials

Six floor materials (linoleum, a small rug, a water-affected small rug, an area rug, a thick carpet, and a thin carpet) and two bedding materials (a pillow and a mattress), each at least 0.25 m^2, were collected from six flood-affected homes in New Orleans. The homes with at least ~3 ft flood water level were selected and six different types of materials were randomly collected to accommodate typical floor and bedding materials commonly used in a household. Homes were not renovated and ceiling and wall materials were excluded because they were not readily available from the owners. The materials were collected approximately 1 year after Hurricane Katrina, however, they were determined to have moisture incursion and thus were expected to have fungal and bacterial contamination. Environmental characteristics of these homes are summarized in Table 1. Two types of indoor carpets, with considerably different fiber lengths, were collected. The thicknesses of the thick and thin carpets were 15 and 9 mm, respectively, and both were made of synthetic fibers. The water-affected small rug and the mattress had some moisture at the time of collection and upon conduction of the experiments. Other materials were also affected by flood water; however, they were dry and covered with sediment and dust at the time of collection.

The material samples were collected in clean airtight plastic bags after cutting from the surfaces (if applicable) with appropriate biosafety precautions. Immediately after collection from homes, the samples were sent to the University of Cincinnati and were preserved in laboratory incubators at approximately 25 °C and 51–72% RH until experiments could be conducted (2–3 weeks post-collection). It was decided to store the material samples at room temperature to avoid moisture condensation on the material surfaces, as this could affect natural aerosolization potential of biocontaminants.

2.2. Study of the aerosolization of biocontaminants from flood-affected materials via FSSST

As stated above, the aerosolization of viable and total fungi, (1→3)-β-D glucan, and endotoxin were studied using the FSSST. The FSSST sampling unit is a closed aerosolization chamber, equipped with two pumps, that was secured on the flood-affected contaminated material surface during aerosolization tests. A push vacuum pump produces the airflow that passes through the HEPA filter (1244 HEPA capsule filter; PALL Gelman Laboratory, Ann Arbor, MI, USA). The incoming air flow Q_{IN} (12 L/min), directed through the 112-orifice stage at the bottom of the device, creates air jets toward the contaminated surface; the biocontaminants are aerosolized by the air jets and were collected into a BioSampler (SKC, Inc., Eighty Four, PA, USA) (see sampling set-up—I in Fig. 1) connected with the FSSST set-up through a vinyl tubing. The process inside the FSSST cap simulates the release of biocontaminants from the surface, where they have been accumulate, with their subsequent (immediate) collection into the BioSampler. The detailed design and performance characteristics of the FSSST have been presented elsewhere (Grinshpun et al., 2002; Sivasubramani et al., 2004a; Górny et al., 2002).

The BioSampler was located at the outlet of the FSSST and operated by a pull vacuum pump at $Q_{SAMPLE} = 12.5$ L/min. The collection vessel of the BioSampler was filled with a 20 mL suspension of pyrogen free, sterilized water mixed with 0.05% Tween 80. To minimize contamination of ambient air, the flow rate balance was set so that Q_{IN} was slightly lower than Q_{SAMPLE} ($\Delta = 0.5$ L/min). The device was thoroughly cleaned between the tests with 70% ethyl alcohol, and a separate sterile BioSampler was used for each test. After sampling, the collection fluid from each BioSampler was divided into aliquots. One aliquot was immediately used for culturing fungi, and the other aliquots were stored at −20 °C for the analyses of (1→3)-β-D-glucan, endotoxin, and total fungi (by microscopy).

All aerosolization tests were conducted inside a Class II Biosafety cabinet. Three replicate samples were collected from different areas of each material

Table 1
Characteristics of flood-affected homes and locations from where eight floor and bedding materials were collected.

Material	Home type	Room type	Flood-water level (feet)	Temperature (°C)	Relative humidity (%)	Visible mold
Linoleum and small rug	Wood frame and hardwood floor	Living room and kitchen	~3	23.8	54.2	Only linoleum had several dry spots
Small rug (water affected) and pillow	Wood frame and hardwood floor	Living room and kitchen	~3	21.8	67.8	No
Area rug	Brick ranch home on slab; floor with hardwood, tile, terrazzo, and area rug	Central living area	~10	10.5	34	Several dry spots near the margins
Thick and thin carpets	Raised double (shotgun style); wood flooring in bedrooms, carpet in living room and master bed room	Living room	~5	20.0	31	No
Mattress	Raised double (shotgun style) with wood frame, wood flooring throughout with tile in kitchen and bathroom	Bedroom	~10	22.7	46	A few black spots indicating active mold growth

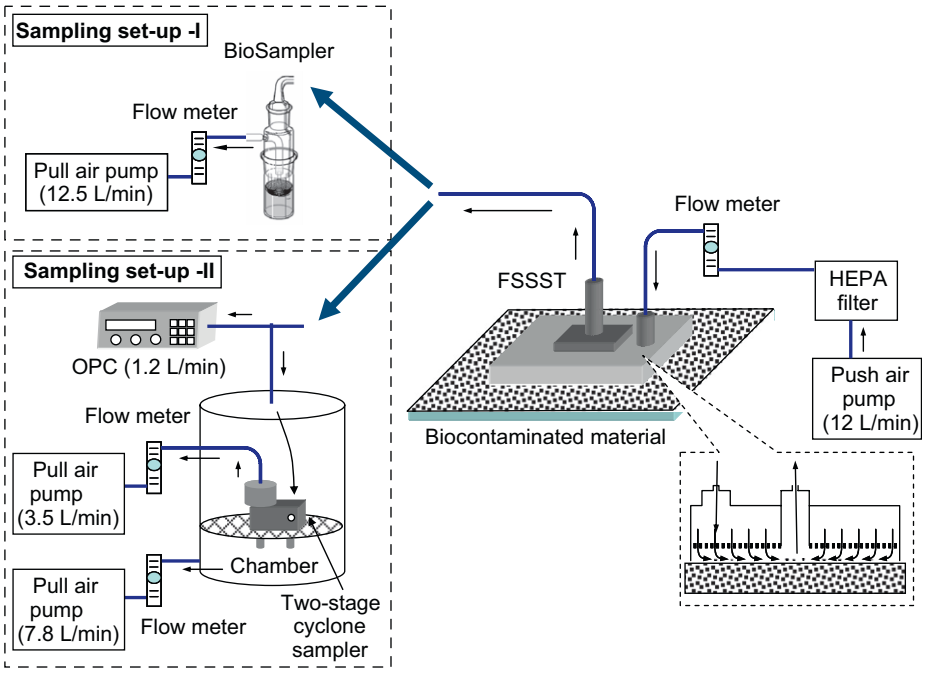

Fig. 1. Experimental set-up to test the aerosolization of biocontaminants (set-up—I) and size-selective measurement of aerosolized biocontaminants (set-up—II).

(randomly selected approximately 0.25 m^2 of the surface area), and appropriate blanks (samples from sterile aluminum foil surfaces) were collected as well. Each replicate consisted of samples collected for 5, 10, and 20 min consecutively from the same sampling spot and cumulative biocontaminant levels of 5, 15 (= 5+10), and 35 (= 5+10+20) min were used for data analysis.

Bioaerosol concentrations are normally expressed as an amount per cubic meter of air. However, in this study, to represent *aerosolizable* biocontaminants, their amounts were expressed per square meter of the area from which they were aerosolized. This allowed for the generation of the data that can be compared to those obtained from vacuum samples. By characterizing the release rate, one can calculate the concentration of the biocontaminants applying specific air volume and ventilation rate for the room. These results should be considered as the maximum aerosolizable concentration level of each selected biocontaminant in indoor air.

2.3. Collection of culturable fungi from material surfaces by swabbing and extraction

For comparison, two additional methods were also applied for the analysis of culturable fungi. The first was swab sampling of 1 cm^2 surfaces with wetted (using sterile distilled water) sterile cotton wood-tipped sticks, following the protocol described by Hung et al. (2005). The second was extraction of 1 cm^2 pieces of contaminated materials in 10 mL de-ionized sterile water with 0.05% Tween 80 using a touch mixer (model 231, Fisher Scientific, Pittsburgh, PA, USA) for 2 min and ultrasonic bath (FS20, Fisher Scientific) agitation for 10 min. The water in the ultrasonic bath was replaced between sonication of individual samples to avoid the temperature increase.

2.4. Particle size-selective study of (1→3)-β-D glucan and endotoxin using the NIOSH two-stage cyclone sampler combined with FSSST

The NIOSH two-stage cyclone sampler consists of two screw-top 1.5 mL microcentrifuge tubes (Model no. 506-624: PGC Scientifics Corp., Frederick, MD) and a 37-mm filter holder with 0.8 μm polycarbonate filter (SKC Inc.) (see sampling set-up—II in Fig. 1) connected with the FSSST set-up through a vinyl tubing. At an air flow rate of 3.5 L/min, the 50% cut-off diameters of the first and second tubes are 1.8 and 1.0 μm, respectively. Thus, the sampler collects aerosolized biocontaminants into three particle size ranges: <1.0, 1.0–1.8, and >1.8 μm. An Optical Particle Counter, OPC (model 1.108, Grimm Technologies, Inc., Douglasville, GA, USA) with a flow rate of 1.2 L/min was operated in parallel to the cyclone sampler to monitor the release stability of aerosolized biocontaminants. The design and performance characteristics of the NIOSH two-stage cyclone sampler were

presented by Lindsley et al. (2006). The size-selective samples were analyzed for (1→3)-β-D glucan and endotoxin content for five selected material samples.

2.5. Collection of dust samples by a vacuum cleaner

Dust samples were collected from the same eight collected materials, using a standardized dust sampling protocol (HUD, 2004), into a small filter bag by vacuuming approximately 0.25 m^2 (adjacent to positions used for FSSST) with a Filter Queen Majestic® vacuum cleaner (Health-Mor, HMI Industries Inc., Seven Hills, OH, USA) for 5 min. Samples were collected on the same day followed by the experiments with FSSST. Dust samples were sieved (355 μm sieve; No. 45; W.S. Tyler, Mentor, OH, USA); the fine dust was divided into sub-samples and stored at −20 °C until the analysis. Immediately before analysis, aliquots of dust were extracted for (1→3)-β-D-glucan and endotoxin, as described below. For dust mite allergen extraction, one aliquot of 100 mg was extracted in 2 mL phosphate-buffered saline (PBS) with 0.05% Tween 20, and incubated at 30 °C on a platform shaker for 1 h. Dust samples were not used for fungal analysis, as spores are masked by dust particles in microscopic spore counting, and preliminary data showed levels of viable fungi below the lower limit of detection (<LOD) in many samples.

2.6. Analysis of culturable fungi

Culture-based analyses were performed for all of the samples collected with the Biosampler, as well as for swab samples and samples extracted directly from selected materials. The extracts were serially diluted and plated on 2% malt extract agar (MEA; Difco Laboratories, Detroit, MI, USA) and dichloran-18-glycerol agar (DG18; Oxoid, Basingstoke, Hampshire, UK) with chloramphenicol antibiotic at a concentration of 100 mg/L. The plates were incubated at 25±2 °C for 7 days. Blank media and blank buffer cultivations were also conducted for quality control. Colonies were identified to genus, based on gross morphology and spore-forming structures; high-resolution light microscopy was used (Labophot 2, Nikon Corp., Tokyo, Japan), following the illustrated identification manuals by Smith (1990) and Ellis (1971) and based on reference slides (Aerobiology Instruction and Research, Brookline, MA, USA). The confirmed colonies were enumerated, and concentrations were expressed as aerosolized CFU per m^2 of contaminated surfaces.

2.7. Microscopic analysis of total fungi

An aliquot of the BioSampler extract was filtered through a 13-mm mixed cellulose esterase filter (Millipore Corp., Bedford, MA, USA). Each filter was placed onto a slide and allowed to completely dry. Once the filter was dry, the filter was made clear through treatment with acetone vapor, as described by Adhikari et al. (2003). Fungal spores in all air samples were counted on the filter in 40 microscopic fields using a bright light microscope (Labophot2, Nikon Corp., Japan) at a magnification of 400× or 1000×, if required. Fungal spores were identified morphologically to genus/group level (following the same manuals and reference slides stated above), and the results were expressed as aerosolized spores/m^2.

2.8. Analysis of (1→3)-β-D glucan

The (1→3)-β-D-glucan concentrations in different extracts were analyzed using the kinetic chromogenic *Limulus* Amebocyte Lysate (LAL) assay (Glucatell, Associates of Cape Cod, East Falmouth, MA, USA). This assay has been successfully used for analysis of (1→3)-β-D-glucan, in both air and dust samples by the University of Cincinnati group (Lee et al., 2006; Iossifova et al., 2007). Protocols used in the referenced investigations were followed in the present study. The Biosampler suspension of 0.5 mL was treated with 0.5 mL of 0.6 M NaOH and extracted for 1 h. For dust samples, 25 mg dust was extracted in 1.0 mL of 0.6 M NaOH for 1 h, followed by centrifugation at 7000 rpm (5204g) for 1 min. The supernatant liquid extracts were used for (1→3)-β-D-glucan analysis. Aerosolized (1→3)-β-D-glucan concentrations were presented in ng/m^2.

2.9. Analysis of endotoxin

Endotoxins in different extracts were analyzed using the endotoxin-specific kinetic chromogenic LAL-assay (Pyrochrome, Associates of Cape Cod). In total 0.5 mL of liquid extracts from BioSamplers were sonicated for 1 h at room temperature, with shaking for 15 s every 15 min using a touch mixer (Fisher Scientific). For dust samples, 25 mg of dust was extracted in 1.0 mL pyrogen-free sterile water for 1 h by sonication and intermittent shaking, followed by centrifugation at 7000 rpm for 1 min. Supernatants were collected and analyzed as stated above. Endotoxin results were presented in EU/m^2.

2.10. Analysis of dust mite allergens

Dust extracts were serially diluted in phosphate-buffered saline with 1% bovine serum albumin and 0.05% Tween 20 (BSA-PBS-T) at pH 7.4. Two-site monoclonal antibody (MAB) sandwich ELISAs for Der p 1 and Der f 1 were used (Indoor Biotechnologies, Inc., Charlottesville, VA, USA) as described by Luczynska et al. (1989). The LOD for both Der p 1 and Der f 1 analyses by this method was 0.025 μg/g.

2.11. Statistical analysis

Normal distributions of the data were checked by Shapiro–Wilk and Kolmogorov–Smirnov tests. Analysis of variance (ANOVA) and Kruskal–Wallis tests were conducted to compare normally-distributed and non-normally distributed data, respectively, for different collection times and size fractions. Scheffe and Bonferroni post-hoc tests were conducted to understand which groups differ for ANOVA and Kruskal–Wallis tests (at $P = 0.05$ level of significance), respectively. Non-parametric Spearman's correlation coefficients were calculated to check the correlation between the aerosolized (1→3)-β-D-glucan and endotoxin levels, when the data were not normally distributed. Values <LOD were replaced by LOD/2 in the statistical analyses. All statistical tests were performed using the SPSS 11.0 for Windows (SPSS Inc., Chicago, IL, USA) and SAS/Stat 9.1 (SAS Institute Inc., Cary, NC, USA) softwares.

3. Results

3.1. Aerosolization of culturable fungi

As presented in Table 2, the average levels of culturable fungi aerosolized from different contaminated materials ranged from <LOD to approximately 2.59×10^5 CFU/m^2. The highest culturable fungi concentration was observed when examining aerosolization from the contaminated mattress, which was slightly moist at the time of collection. Colonies of *Aspergillus*, *Penicillium*, and *Cladosporium*, along with non-sporulating colonies were detected in different samples.

With the linoleum, the small rug, and the pillow excluded (as their levels of aerosolized culturable fungi were <LOD), the culturability ranged from 3% (area rug) to 41% (mattress) [culturability % = concentration of culturable fungi/concentration of total fungi × 100]. Since the colony counts were <LOD in many aerosol samples, culturable analysis was also performed for fungi collected by swabbing and extraction from materials such as linoleum and mattress. These samples represented extremes with respect to the levels of aerosolized culturable fungi. For the mattress, swabbing provided a similar concentration (1.6×10^5 CFU/m^2) of viable fungi to that determined with the FSSST, while extraction provided an approximately 10-fold higher concentration (2.0×10^6 CFU/m^2). Extraction and swabbing from linoleum provided culturable fungi concentrations of 5.0×10^5 and 4.0×10^4 CFU/m^2, respectively.

3.2. Aerosolization of total fungi

Since a considerable number of samples exhibited low levels (<LOD) of aerosolized culturable fungi, only 33% of the FSSST samples (one sample out of every three replicates for each material was randomly chosen) were analyzed for total fungi. The results on aerosolized total fungi (a cumulative level for 35 min) and individual genera/classes are presented in Tables 2 and 3. In addition to the genera presented in Table 3, we found sporadic presence of *Arthrinium*, *Ganoderma*, *Polythrincium*, and *Torula* spores. The concentrations of total aerosolized fungi in different materials ranged approximately from 2.07×10^5 to 1.6×10^6 spores/m^2.

When log-transformed cumulative levels of total aerosolized fungi for 8 materials collected during consecutive 5, 10, and 20 min (cumulative 5, 15, and 35 min) were compared using

Table 2
Biocontaminants aerosolized from floor and bedding materials collected in flood-affected homes of New Orleans using FSSST (cumulative levels over 35 min).

Material	Culturable fungi ($\times 10^3$ CFU/m^2)	Total fungi[a] ($\times 10^3$ spores/m^2)	(1→3)-β-D glucan ($\times 10^3$ ng/m^2)	Endotoxin ($\times 10^3$ EU/m^2)
Linoleum	<LOD	475	18.25±23.57	59.15±70.45
Small rug	<LOD	259	28.54±32.94	93.09±141.52
Small rug (water affected)	8±8	615	21.56±26.45	9.86±4.16
Pillow	<LOD	710	13.12±8.90	8.39±4.91
Area rug	25±22	1145	12.18±7.40	37.37±30.28
Thick carpet	8±8	207	3.43±1.90	0.70±0.58
Thin carpet	11±19	324	2.00±2.26	0.81±0.31
Mattress	259±353	1598	2.01±0.64	11.52±4.81

Note: values represent cumulative data over three consecutive sampling time periods of 5, 10, and 20 min ($t = 35$ min). Three replicates ($n = 3$) were averaged and mean±SD data are presented, except for total fungi. <LOD = no detectable CFUs in the sample.
Lower limit of detections (LOD): culturable fungi: 8264 CFU/m^2; total fungi: 5587 spores/m^2; (1→3)-β-D-glucan: 8.39 ng/m^2; endotoxin: 86.76 EU/m^2.
[a] Total fungi were analyzed in 33% samples only ($n = 1$).

Table 3
Composition of fungal spores aerosolized from floor and bedding materials collected in flood-affected homes of New Orleans ($\times 10^3$ spores/m^2).

Material	Linoleum	Small rug	Small rug (water-affected)	Pillow	Area rug	Thick carpet	Thin carpet	Mattress
Fungal genera/class								
Alternaria	6	6	6	6	<LOD	<LOD	<LOD	<LOD
Ascospores	17	<LOD	39	39	28	11	6	<LOD
Aspergillus/Penicillium	369	199	520	564	927	151	212	469
Basidiospores	<LOD	11	6	11	<LOD	<LOD	<LOD	<LOD
Botrytis	11	6	6	<LOD	6	<LOD	<LOD	<LOD
Chaetomium	<LOD	6	<LOD	<LOD	<LOD	<LOD	<LOD	61
Cladosporium	34	<LOD	22	28	45	<LOD	6	<LOD
Stachybotrys	<LOD	6	6	<LOD	39	6	22	1034
Unknown	39	22	11	61	101	39	61	34
Total spores	475	260	615	710	1145	207	324	1598

one-way Analysis of variance (ANOVA) in all 8 materials, a significant difference was found ($P<0.05$) between sampling periods. Scheffe post hoc test, however, showed a difference only between the levels of total aerosolized fungi collected at 5 and 35 min.

3.3. Aerosolization of (1→3)-β-D glucan

The range of (1→3)-β-D glucan aerosolized from different materials ranged approximately from 2.0×10^3 to 2.9×10^4 ng/m^2 (mean values, Table 2). When aerosolization for cumulative periods of 5, 15, and 35 min were compared, there was a slight increase of aerosolization with time (Fig. 2). However, this was not statistically significant in most samples ($P>0.05$ in ANOVA and Kruskal–Wallis tests). Only for the thick carpet was a significant difference observed, and post hoc multiple comparisons indicated that the concentration at 5 min differed significantly from the concentration at 35 min. A large variation in the aerosolized (1→3)-β-D glucan levels was observed for the three replicate samples collected from different positions from the same materials [coefficient of variation (CV) = 60–129%].

Size-selective data on (1→3)-β-D glucans aerosolized from different materials are presented in Fig. 3. There were no significant differences among the size fractions of <1.0, 1.0–1.8, and >1.8 μm for three of the five tested materials ($P>0.05$; ANOVA and Kruskal–Wallis tests). In the case of the linoleum and the mattress, a significant difference was observed ($P<0.05$; Kruskal–Wallis tests). The Bonferroni post-hoc test revealed that the size fraction >1.8 μm was different from the size fraction of <1 μm for linoleum and from the size fraction of 1–1.8 μm for mattress.

3.4. Aerosolization of endotoxin

Endotoxin aerosolized from different materials ranged from 7.0×10^2 to 9.3×10^4 EU/m^2 (mean values, Table 2). There was a slight increase of aerosolization from all materials with time (Fig. 4); however, the differences were not statistically significant ($P>0.05$; ANOVA and Kruskal–Wallis tests). Similar to (1→3)-β-D glucan, large variations of aerosolized endotoxins were observed in samples collected from different positions of the same material (CV = 38–119%).

The size-selective data on the aerosolized endotoxin are presented in Fig. 5. Higher concentrations were observed for the larger fraction (>1.8 μm) than for the other two; however, no statistically significant differences were found.

3.5. Correlation between aerosolized (1→3)-β-D glucan and endotoxin

Non-parametric Spearman's correlation coefficient between the data on aerosolized (1→3)-β-D glucan and endotoxin collected from all materials were calculated for all three sampling periods. A significant positive correlation was observed between the two data sets (correlation coefficient = 0.425; $P<0.001$).

3.6. Surface (1→3)-β-D glucan and endotoxin investigated with a vacuum cleaner

In all cases, except for the pillow, the vacuum cleaner sample showed higher levels of (1→3)-β-D glucan and endotoxin than the FSSST (Table 4). The average ratios (vacuum sample to FSSST sample) were 113±132 for the glucan and 4594±8022 for the endotoxin (mean±SD). The levels of (1→3)-β-D glucan and

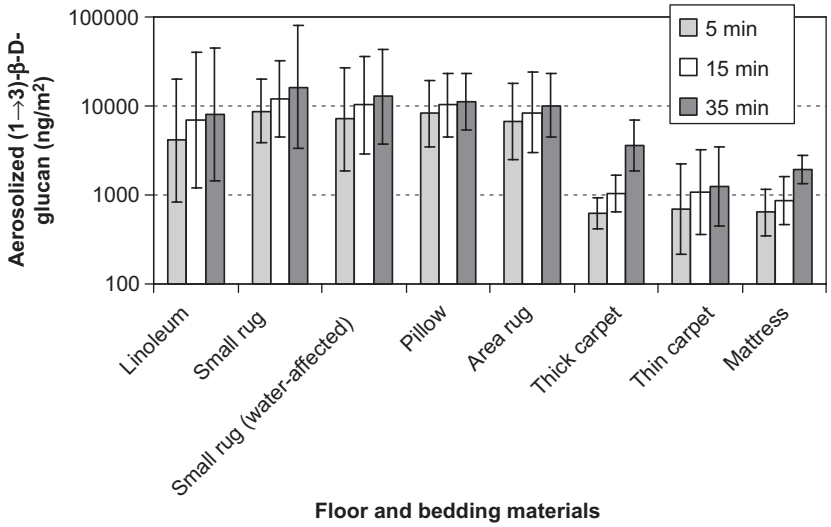

Fig. 2. (1→3)-β-D-glucan aerosolized from contaminated materials collected in flood-affected homes of New Orleans. The histograms present geometric mean of three replicates and error bars indicate geometric standard deviations.

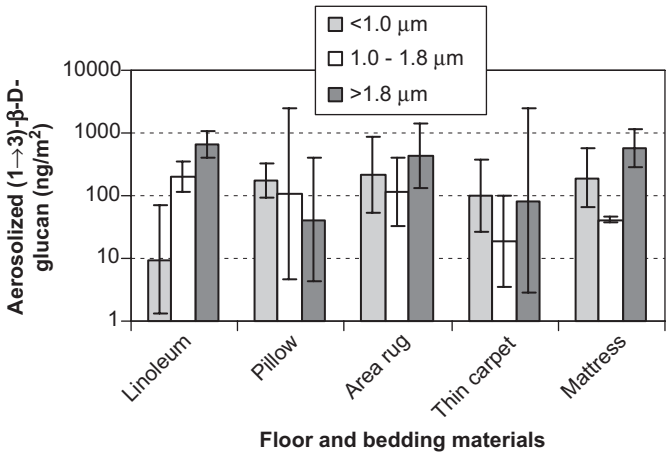

Fig. 3. (1→3)-β-D-glucan aerosolized from different floor and bedding materials collected in flood-affected homes of New Orleans (presented size-selectively). The histograms present geometric mean of three replicates and error bars indicate geometric standard deviations.

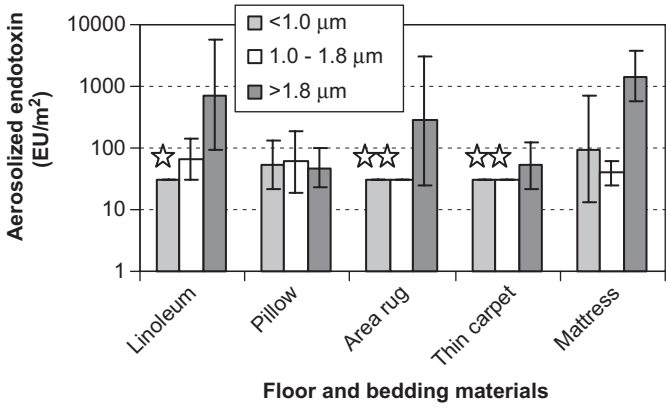

Fig. 5. Endotoxin aerosolized from different floor and bedding materials collected in flood-affected homes of New Orleans (presented size-selectively). The histograms present geometric mean of three replicates and error bars indicate geometric standard deviations. Star signs indicate <LOD data points.

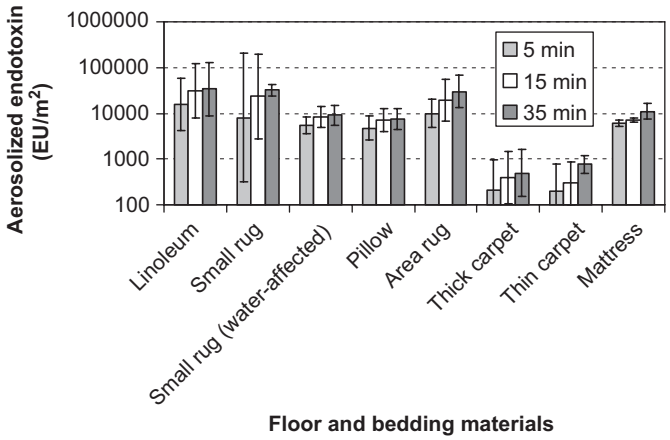

Fig. 4. Endotoxin aerosolized from contaminated materials collected in flood-affected homes of New Orleans. The histograms present geometric mean of three replicates and error bars indicate geometric standard deviations.

endotoxin collected with the vacuum cleaner did not show any significant correlation with the corresponding data obtained with the FSSST [for (1→3)-β-D glucan: $r = 0.143$; $P = 0.736$; for endotoxin: $r = -0.405$; $P = 0.320$]. It should be noted that the FSSST data in Table 4 represent the samples generated by the first 5-min sampling to relate these to the vacuum samples collected over the same time interval.

3.7. Levels of dust mite allergens

The collected amounts of dust mite allergens (both Der f1 and Der p1) were below the detection limit in all FSSST samples. Following this finding, additional experiments were performed. Approximately 200 mg of dust mite allergen-containing dusts (supplied by Healthy Housing Solutions, Inc., Columbia, MD, USA) were spread on a 121 cm^2 area, and then samples were collected from the area using the FSSST. All nine samples analyzed from this additional experiment also failed to show any detectable levels of dust mite allergens. Of the dust samples collected by vacuum

Table 4
Comparison of aerosolized (1→3)-β-D-glucan and endotoxin levels collected by FSSST (first 5-min samples) and a vacuum cleaner.

Materials	Aerosolized (1→3)-β-D-glucan ($\times 10^3$ ng/m^2)			Aerosolized endotoxin ($\times 10^3$ EU/m^2)		
	FSSST (first 5 min); mean ± SD ($n=3$)	Vacuum cleaner ($n=1$)	Ratio (vacuum cleaner: FSSST)	FSSST (first 5 min); mean ± SD ($n=3$)	Vacuum cleaner ($n=1$)	Ratio (vacuum cleaner: FSSST $\times 10^3$)
Linoleum	8.72 ± 11.37	27.47	3	15.26 ± 10.21	1678.81	0.11
Small rug	10.48 ± 6.28	2061.44	197	57.53 ± 99.12	4032.62	0.07
Small rug (water-affected)	13.22 ± 17.02	107.80	8	4.65 ± 4.03	2333.09	0.50
Pillow	10.32 ± 8.25	8.20	0.8	3.05 ± 4.43	1535.57	0.50
Area rug	8.62 ± 5.77	906.47	105	7.12 ± 8.06	820.90	0.15
Thick carpet	0.59 ± 0.33	161.43	273	0.39 ± 0.44	6144.50	15.83
Thin carpet	1.09 ± 1.24	344.24	316	0.31 ± 0.23	5868.17	19.17
Mattress	0.71 ± 0.34	2.04	3	6.09 ± 1.13	2671.82	0.44

cleaners, detectable Der f1 was found only in the pillow (0.38 μg/g) and the small rug (0.4 μg/g); detectable Der p1 was found only in the small rug (1.3 μg/g).

4. Discussion

Results obtained with the FSSST for the levels of aerosolized culturable fungi were above detection limit in five materials, ranging up to $(2.59 \pm 3.53) \times 10^5$ CFU/m^2. Measured levels of total aerosolized fungi in all materials ranged from approximately 2.1×10^5 to 1.6×10^6 spores/m^2. Previously, FSSST had been used in a field study by Niemeier et al. (2006) to investigate aerosolization of culturable and total fungal spores in visibly mold-contaminated homes in Ohio. The quoted study reported approximately 10^5 CFU/m^2 of aerosolized culturable fungi and 10^6 spores/m^2 of aerosolized total fungal spores. While generally of the same order of magnitude, for several materials, the aerosolized concentration levels obtained in the present study were lower than those reported by Niemeier et al. (for both the culturable and total fungi). It should be noted that Niemeier et al. selected particularly moldy wall surfaces for the FSSST application, whereas we applied a random selection of sampling surfaces, irrespective of the signs of visible mold or water damage. The levels of culturable fungi aerosolized in our tests with the mattress (which was slightly moist at the time of collection) were comparable to those reported in Niemeier's study.

We found levels of aerosolized culturable fungi in many of the FSSST samples, consistently below the lower limit of detection. However, based on our observations of culturable fungi levels on surfaces by swabbing and extraction, as well as previously-measured aerosolization ratios for fungal spores [1–2% for *Aspergillus/Penicillium* as reported in Sivasubramani et al. (2004b)], detectable levels of aerosolized culturable fungi were expected in most samples. These expectations were unmet, potentially due to: (a) mud or sediment dust layers inhibiting germination, and/or (b) varying species of fungi present in different materials with disparate culturability in the selected culture media.

Culturable fungi were identified up to the genus level, and colonies of three fungal genera were identified: *Aspergillus*, *Penicillium*, and *Cladosporium*, along with non-sporulating colonies. We found that swabbing provided a similar concentration of viable fungi to that determined with the FSSST for the mattress, in contrast to the similar previous comparison made by Niemeier et al. (2006). Although swabbing is routinely used for environmental sampling, the levels of collected viable fungi using swabbing may not be as appropriate as FSSST, particularly for the porous mattress surfaces. While analyzing aerosolized total fungal spores, twelve fungal genera and classes were identified; *Aspergillus/Penicillium* was the most prevalent in all samples, except for the mattress (Table 3). The concentration of *Stachybotrys*, however, was highest in the mattress, which was moist at the time of collection. Similar to Niemeier et al. who utilized the FSSST for enumerating total fungal spores in field samples, we also observed common occurrences of *Aspergillus/Penicillium*, *Cladosporium*, Ascospores, *Chaetomium*, and *Stachybotrys*. Additionally, we frequently found the presence of *Alternaria*, *Botrytis*, and Basidiospores. While comparing the occurrence of culturable fungi with Niemeier et al.'s study, we found significantly lower species variability. In addition to *Aspergillus*, *Penicillium, Cladosporium*, and non-sporulating colonies (identified in the present study), Niemeier et al. also recorded colonies of *Mucor, Paecilomyces, Pithomyces, Stachybotrys*, and *Epicoccum* in FSSST samples. Among different fungal genera that we isolated in the present study, *Cladosporium*, *Aspergillus*, *Penicillium*, and *Alternaria* have been strongly associated with allergic respiratory disease, especially asthma (Douwes et al., 2003). The relationship between increased risk of building-associated pulmonary disease and the presence of *Stachybotrys* in bulk surface samples has been reported by several investigators (Hodgson et al., 1998).

This investigation is the first one that utilized the FSSST with the field samples for quantifying the aerosolization of (1→3)-β-D glucan and endotoxin. The average levels of aerosolized (1→3)-β-D glucan and endotoxin in all materials were approximately 2.0×10^3–2.9×10^4 ng/m^2 and 7.0×10^2–9.3×10^4 EU/m^2, respectively. In a recent study by this group (Seo et al., 2008) the FSSST was used to characterize the aerosolization of (1→3)-β-D glucan in the laboratory from ceiling tile and gypsum board samples artificially contaminated by *Aspergillus versicolor* and *Stachybotrys chartarum*. The reported concentrations of aerosolized glucan ranged from 2.0×10^0 to 1.6×10^4 ng per material sample (area of 60 cm^2), which corresponds to the range 3.3×10^2–2.7×10^6 ng/m^2. The elevated glucan concentration range observed by Seo et al. can be explained by the difference in the initial contamination levels of materials used in these two studies.

Vacuuming has traditionally been used for the collection of (1→3)-β-D glucan and endotoxin in field studies. The levels of (1→3)-β-D glucan obtained in this study, by vacuuming five flood-affected materials obtained in New Orleans homes (ranging from 2.04×10^3 to 2.061×10^6 μg/m^2), were generally higher than those determined from the vacuum-collected dust samples in 574 Cincinnati homes in a report recently published by Iossifova et al. (2007) (geometric mean = 18.4 μg/m^2). Similarly, the endotoxin levels in all materials collected by vacuuming (8.21×10^5–6.144×10^6 EU/m^2) were approximately 10^3–10^5 times higher than in dust samples collected from homes in New York (3,892 EU/m^2; Perzanowski et al., 2006) and Cincinnati (24 EU/m^2; Iossifova et al., 2007).

The amounts of dust mite allergens in all FSSST samples collected from different materials were below the detection limit. We assumed that dust mites either rarely infested flood-affected materials or their allergens were altered in some way that precluded measurement. Interestingly, the materials, which aerosolized very low amounts of culturable fungi (including those <LOD), produced dust samples (collected by vacuuming) with detectable levels of dust mite allergens. This finding suggests that an antagonistic ecological relationship between dust mites and species of fungi present in these materials is possible. Since we could not detect aerosolizable dust mite allergens in most samples, their potentially inhibitory roles in fungal aerosolization and stimulatory roles in aerosolization of (1→3)-β-D glucan have to be investigated.

Standardization of sampling time is important while characterizing aerosolized microorganisms and hazardous microbial substances using a new technique. Since the application of FSSST is not yet conventional, and no sampling protocol is available for prospective future field studies, we conducted a series of experiments here to compare the aerosolized biocontaminant levels collected during cumulative 5, 15, and 35 min with the FSSST. For the level of total aerosolized fungi and (1→3)-β-D glucan, the results obtained at 15 min did not significantly differ from 5 to 35 min levels. For endotoxin, no differences were found between the results obtained at the three sampling times. These observations indicate that FSSST operation for 15 min would be sufficient to collect aerosolizable total fungi and (1→3)-β-D glucan from the surfaces of flood-affected materials, and a shorter 5 min sampling period can be considered for collecting aerosolized endotoxin.

When comparing the aerosolized biocontaminant levels among different materials, we found wide inter- and intra-sample variations. The moist mattress had the maximum levels of culturable and total fungi; however, there must be other factors for the growth of culturable fungi besides moisture, such as availability of nutrients and growth-supporting materials. These two factors may also influence the variability of fungal flora on surfaces and interactions between various species. Unlike with the mattress, we found only a few culturable fungi aerosolized from the water-affected small rug, which was moist at the time of collection. Higher concentrations of aerosolized (1→3)-β-D glucan were observed in small rugs and linoleum, and lower concentrations were found in the mattress. The latter is particularly interesting, since the highest levels of culturable and total fungi were aerosolized from the mattress. These contrasting observations indicate that the major sources of (1→3)-β-D glucan could be different from viable and non-viable fungi. Fragmented fungal mycelium or fungal spore-bearing structures, plant material or other sources (unknown at the time) may have been among these sources. Different species of fungi may have different (1→3)-β-D glucan content (Fogelmark and Rylander, 1997). As we found higher concentrations of *Stachybotrys* and *Chaetomium* in the mattress and, in contrast, *Alternaria* and *Cladosporium* in linoleum and small rugs, the data point to a difference in primary contaminant fungal species. Higher concentrations of aerosolized endotoxin were observed in small rugs and linoleum and lower concentrations were found in carpets, pillow, and mattress. The moist small rug did not show an elevated level of endotoxin, indicating that water activity may not be directly related to endotoxin levels. Large variations of (1→3)-β-D glucan and endotoxin in different samples can be attributed to the different levels of fungal and bacterial growth, depending on moisture availability or sediment deposition. Large variations were also observed from the different positions of the same samples, which may be related to different levels of water damage or availability of organic nutrients in different portions of the materials. These observations should be important for the future field studies in flood-affected and water-damaged homes. We found a significant positive correlation between the aerosolized (1→3)-β-D glucan and endotoxin levels. This is consistent with several previous studies (Schram-Bijkerk et al., 2005; Douwes et al., 2006; Iossifova et al., 2007). As stated in the introduction, floodwater sediment with decomposing organic materials can support growth of microorganisms, including fungi and bacteria, equally. Thus, this positive significant correlation between the aerosolized (1→3)-β-D glucan and endotoxin levels is feasible.

This study gives unique new size-selective data on the aerosolization of microbial components. We found that the size fractions of <1 μm and 1–1.8 μm have comparable levels of (1→3)-β-D glucans and endotoxin to the one determined for >1.8 μm in most of the materials. This is an important finding because it suggests that smaller respirable particles may have levels of (1→3)-β-D glucan and endotoxin as high as larger particles; this raises considerable concern regarding exposure to small-size biocontaminants, which can be aerosolized in flood-affected homes. These observations also indicate that smaller particle size fractions of microbial biocontaminants contribute significantly to the total inhalation exposures. Since submicron fungal fragments can be a major source of (1→3)-β-D glucan, the above finding has strong implications in the respiratory health effects of fungi. The effect of muddy flood water damage on the microbial growth in our selected materials may be different than regular moisture damage in homes; however, we believe these findings have general significance and would be applicable for the release of (1→3)-β-D glucan and endotoxin from moist surfaces.

Higher levels of (1→3)-β-D glucan and endotoxin were obtained in most dust samples collected with the vacuum cleaner, as compared to the levels measured with the FSSST (exceeding 10^2 fold in some samples for (1→3)-β-D glucan and ranging from about 10^2 to $>10^4$ fold for endotoxin). A contrasting result was observed only for the pillow, when glucan in dust was slightly lower than in the FSSST sample. The soft surface of the pillow may have blocked the vacuum cleaner nozzle and inhibited collection. The high magnitude of the differences and the lack of correlation between the FSSST and vacuum cleaner data suggest that a vacuum cleaner can overestimate inhalation exposure risks of biocontaminants. Since a vacuum cleaner collects both the aerosolizable biocontaminants and those adhering to the surface, these findings are explainable.

The present study allowed for the characterization of aerosolizable fungi, (1→3)-β-D glucan, and endotoxin per square meter area (overall and in different particle size fractions) in the worst case situation, when the materials were collected from homes heavily affected by a major water damage. One of the challenges of this study was associated with the large coefficient of variations in biocontaminants' levels observed in different materials. This suggests that more flood-affected materials must be studied, and more repeat samples should be collected from different positions to achieve an enhanced understanding of the collective aerosolization strengths of these biocontaminants in flood-affected homes. The results of the study, pertaining to the aerosolization of fungi, (1→3)-β-D-glucan, and endotoxin, as well as the size-selective data for the above factors will supplement the previous findings of Chew et al. (2006) and Rao et al. (2007), who also have investigated airborne concentration levels of these biocontaminants in flood-affected homes of New Orleans. Chew et al. (2006) found culturable fungi levels ranged from 22,000 to 515,000 colony-forming units (CFUs)/m^3, fungal spore counts were between 82,000 and 630,000 spores/m^3, and endotoxin levels ranged from 17 to 139 endotoxin units (EU)/m^3. Rao et al. (2007) reported rather high concentrations of airborne fungi, endotoxin, and (1→3, 1→6)-β-D-glucans in New Orleans homes,

as well as in outdoor locations after Hurricanes Katrina and Rita. The air sampling time, however, was very short in the quoted studies: 1–20 min and 36–144 min, respectively. It is not known how adequately the biocontaminant exposure levels are represented by these short periods of air sampling. Our approach was to estimate the worst case scenario corresponding to particularly intense aerosolization of biocontaminants. By using the FSSST, this can be achieved with short sampling periods of $\leqslant$20 min. Moreover, the quantitative characterization of source strength of different flood-affected materials for aerosolizing these biocontaminants was beyond the scope of studies conducted by Chew et al. and Rao et al. Overall, the data reported in these two papers, which are considerably complementary to the findings of this study, provide a better understanding of the exposure and inhalation risks to fungi, (1→3)-β-D-glucan, and endotoxin in flood-affected homes in New Orleans.

This study is characterized by some limitations that can be addressed in future investigations. First, a limited number of replications resulted in a rather wide intra-sample variation in the biocontaminant levels. Second, while the choice of materials for testing accounted—among other factors—for their typical use in a household, we acknowledge that some of the tested materials (especially highly-contaminated) may be discarded by the residents during restoration and thus will not anymore represent a source of long-term exposure for the occupants. A follow-up comparative study of restored homes would be appropriate to better address the relevance issue. Furthermore, stratification of biocontaminated materials based on their composition would be useful in future studies because synthetic and organic matters may have different abilities of supporting microbial growth under the same environmental conditions.

The present investigation has several practical implications for further field studies involving techniques for assessing the aerosolization of fungi and other flood/moisture-related indoor biocontaminants from surfaces. It has been proven that the FSSST can efficiently release aerosolizable fungi, (1→3)-β-D glucan, and endotoxin from contaminated surfaces over a relatively short sampling time. A period of 5 min is recommended for field sampling of endotoxins, while 15 min seems to be appropriate for fungi and (1→3)-β-D glucan. Although vacuum cleaners are routinely used for indoor testing, our results indicate that they can overestimate the inhalation exposure risks of aerosolizable surface biocontaminants; parallel investigations of surface biocontaminants, using the FSSST, are highly desirable. The aerosolization rate and size-selective characteristics of fungi, (1→3)-β-D glucan, and endotoxin aerosolized from different materials, which have been quantified in this study, can be used in further exposure assessment research, particularly for estimating exposures to submicron aerosol fractions of these biocontaminants. Our results imply that the monitoring of sub-micron fractions of different microbial biocontaminants and their potential toxicity, followed by a contaminated building renovation, is required to ensure satisfactory control of microbial air pollution in flood-affected buildings. In addition, these results will be useful for developing guidance on respiratory protection against fungal spores during mold remediation. Overall, the information generated in this study is important with respect to restoration and rejuvenation of the flood-affected areas of New Orleans. According to the US Department of HUD, the estimated number of homes in Orleans parish that received 2 ft or more of floodwater after Hurricane Katrina was 103,513 out of 215,101 (HUD, 2007). As with a majority of these homes, those selected for this investigation remained untreated, with mold, dust and water damage visible on the walls and floors. Complete remediation of the flood-affected homes in New Orleans will likely take years, and many policy and logistical decisions in this context have yet to be made. Beyond dealing with consequences of Katrina and Rita, we believe that the lessons learned in this study will be of significant help for assessing burden of microbial contaminants during similar disasters in other regions of the world including major coastal floods from tsunamis, hurricanes, and tornados.

5. Conclusions

In conclusion, significantly higher levels of aerosolized (1→3)-β-D glucan and endotoxin were observed in the flood-affected materials collected in New Orleans, as compared to other studies conducted in urban homes. At the same time, the levels of culturable and total fungi found in these materials were slightly lower than those previously reported for moldy buildings. A significant positive correlation existed between the aerosolized (1→3)-β-D glucan and endotoxin levels. Wide variations of all three biocontaminant levels were observed in different materials. Smaller aerosolized particles ($<$1.8 μm) were found to have the (1→3)-β-D glucan and endotoxin levels comparable to larger ($>$1.8 μm) particles. Since finer particles can be inhaled deeper into airways, this finding raises additional exposure concerns. Short sampling periods of 5 min for endotoxin and 15 min for fungi and (1→3)-β-D glucan were found to be sufficient for detecting most aerosolizable biocontaminants with the FSSST.

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